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NUMBERS AND TYPES OF BACTERIA CARRIED BY CITY FLIES.*

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During the past decade much has been published in regard to the supposed danger from house flies as the disseminators of pathogenic bacteria. More or less evidence has been presented to the effect that these insects may, at times, spread a long category of infections, including, to quote the list of Purdy[1] (see also Hewitt[2]), typhoid fever, infantile diarrhea, bacillary dysentery, cholera, tuberculosis, diphtheria, erysipelas, contagious ophthalmia, cerebrospinal meningitis, anthrax, and possibly smallpox. This evidence has recently been summarized and discussed by Chapin[3] in an interesting manner. For a number of these diseases the chain of evidence is incomplete, but for infections of fecal origin, the guilt of this ubiquitous insect has been clearly established in certain instances, especially as regards typhoid fever. Hamilton,[4] Ficker,[5] Klein,[6] and Bertarelli[7] have severally succeeded in isolating the typhoid bacillus from flies caught in the neighborhood of typhoid fever cases, although the attempts of other investigators under similar circumstances have been fruitless. Hamilton, first, in 1903, was able to prove that an outbreak of typhoid fever, restricted to a certain ward of Chicago where the street sewers were inadequate and the sanitary arrangements of most of the houses were of the worst, was in large measure due to flies acting as carriers of the specific bacilli, which, in fact, were isolated from a number of flies caught in two undrained privies, on the fences and house walls, and in the room of a typhoid patient. In 1910, Bertarelli, in an investigation of 100 flies caught in a household of the better class

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[1] *Jour. Roy. San. Inst.*, 1909, 30, p. 496.

[2] *Quart. Jour. M. Sc.*, 1909, 54, p. 347.

[3] *The Sources and Modes of Infection*, New York, 1910.

[4] *Jour. Am. M. Ass.*, 1903, 40, p. 576.

[5] *Arch. f. Hyg.*, 1903, 46, p. 274.

[6] *Brit. Med. Jour.*, 1908, II, p. 1150.

[7] *Centralbl. f. Bakt.*, I, Orig., 1910, 53, p. 486.

of Italians, who were suffering from an outbreak of typhoid, was successful in the isolation of the typhoid bacillus from the bodies of eight flies. Reed, Vaughan, and Shakespeare[1] have shown that flies were undoubtedly one of the most active agents in the spread of typhoid fever in the U.S. military camps during the Spanish War in 1898.

Although the guilt of the house fly has been clearly established in certain instances as a typhoid spreader, the relative importance of this vehicle of transmission as compared with the other well-known methods of transfer has been by no means clearly established. What is, perhaps, a timely warning of the danger, as regards the popular mind, of over-emphasis being laid on this mode of transmission has recently been voiced by Chapin. He believes that unwarranted faith in the iniquity of these insects may lead to the neglect of the far more serious danger of contact infection, and that a failure of a decrease in the death-rate from typhoid after an enthusiastic anti-fly campaign would tend to bring discredit on the well-grounded warnings of health officers. "It is probable," he concludes, "that under certain conditions, as in military and civil camps, and in filthy communities without sewerage, insects, especially flies, may be an important factor in the spread of fecal-borne diseases, but there is no evidence that in the average city the house fly is a factor of great moment in the dissemination of disease."

With the hope of gaining some additional light on the nature of the bacteria conveyed by flies which frequent a congested part of New York City, I have, during the past summer, investigated in detail the bacterial content of a considerable number of these insects which were caught entering the windows of the laboratory building. The south side of this building faces the rear of a row of tenements of the poorer grade with an intervening space of about 75 feet. The flies caught were among those which were continually circulating in and out of the open windows of these tenements. It seemed possible that the flies might have picked up bacilli of the dysentery type from the soiled diapers of infants sick with infective summer diarrhea, for without doubt such dejecta were occasionally exposed to them. Weather conditions, as it happened, were especially favorable for the work, as in the early part of the summer there was an extended period of exceptional heat and drought.

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[1] *Report on the Origin and Spread of Typhoid Fever in the U.S. Military Camps during the Spanish War,* 1898.

Methods.—The flies, as a rule, were examined in lots of 10. These were caught, without crushing, as they entered the open windows of the laboratory, and placed in a large sterile test tube. They were then shaken for five minutes in 10 c.c. of sterile normal salt solution, and the wash was set aside and labeled I. The flies were then rinsed thoroughly in 20 c.c. of the salt solution, which was drained off and discarded. The washed flies were next placed in 10 c.c. of the salt solution and the abdomens were so squeezed with a sterile platinum spatula that the content of the intestine exuded into the fluid. The thoroughly emulsified intestinal matter of the 10 flies was labeled II.

Platings were made from I and II, suitably diluted, in nutrient agar +1. to phenolphthalein, in litmus lactose agar, and on Conradi-Drigalski medium. These plates were incubated 24 hours at 37° C. The number of colonies on the nutrient agar plates was taken as the total count, although in one instance it was exceeded by the count on the litmus agar plate. The types of colonies which appeared to be dominant were isolated and identified, and in addition a special search was always made for colonies of the dysentery bacillus type. These several types were transplanted to the Hiss semi-solid tube medium and later subjected to cultural and agglutination tests.

In Table 1 are summarized the findings during the course of the summer. The mean temperatures were supplied by the local U.S. Weather Bureau, to whom grateful acknowledgement is made. In this table under I are given the total number of bacteria and the types of sanitary interest found on the surface of the flies; and under II the same data as regards the intestinal content.

The bacterial flora of flies examined in April and in early June included apparently few or no bacilli. It consisted, in fact, as Jackson[1] also found for that season of the year, of cocci commonly existing as saprophytes in nature and of a few molds. The counts in the few instances were low. That this homogeneous and non-fecal flora should persist to the end of spring is rather surprising. The high counts began to appear the last of June, and also an abrupt change in the character of the bacteria, the cocci giving place in large measure to the bacilli characteristic of fecal matter. At the end of the two weeks of excessive heat in July we find the record count, both as regards the number of bacteria on

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[1] *Report to Committee on Pollution .... of Merchants Association of New York*, 1907.

TABLE 1.

Under I are given the total counts and the types of bacteria present on the surface of the flies, and under II the same data for the intestinal content.

DATE	MEAN TEMPERATURE	I			II		
		Total Count	Lactose Fermenters	Types	Total Count	Lactose Fermenters	Types
April 5...	48	330	50 all cocci	Albococcus Aurococcus	4,200	......	All cocci
June 5....	60	540	220 all cocci	Micrococcus flavus Albococcus pyogenes	1,800	800 all cocci	Micrococcus flavus No bacilli
" 9....	72	190	20 cocci	....	10	80	Mostly cocci B. coli communis
" 28...	78	17,000	.....	Streptococcus equinus B. coli communis	42,000	......	....
" 30...	71	6,600	300	Streptococcus fecalis B. coli communior	1,100,000	17,000	Streptococcus fecalis Streptococcus salivarius B. aerogenes
July 7....	79	59,000	1,000	B. coli communis B. acidi lactici	90,000	22,000	B. acidi lactici
" 10...	86	5,400	1,400	B. coli communis B. coli communior	97,500	24,000	Streptococcus fecalis B. coli communior B. acidi lactici
" 13...	78	4,400,000	1,000	B. coli communis	28,500,000	8,000	B. aerogenes
" 25...	70	600,000	1,000	B. lacti acidi	7,500,000	......	B. PARATYPHUS A B. coli communis
" 26...	70	1,400	10	Albococcus pyogenes	16,000	900	Streptococcus fecalis B. aerogenes B. fecalis alkaligenes
" 27...	71	570	10	Streptococcus equinus B. lacti acidi	280,000	200	Streptococcus salivarius B. coli communis B. aerogenes
Aug. 2....	74	17,500	12,000	Streptococcus equinus B. aerogenes B. paracoli Many proteolytic	3,850,000	......	B. fecalis alkaligenes B. paracoli Many proteolytic
" 10...	78	118,000	50,000	B. aerogenes B. paracoli	1,380,000	470,000	B. coli communis B. aerogenes B. acidi lactici B. paracoli
" 14...	76	1,600	0	....	2,700,000	1,700,000	Streptococcus fecalis B. aerogenes B. paracoli
" 17...	80	1,000	0	....	300,000	60,000	B. coli communis B. aerogenes B. paracoli
" 21...	71	810	10	B. xerosis Albococcus pyogenes	38,000	0	....
" 28...	76	84,000	1,000	B. lacti acidi B. paracoli	125,000	4,500	B. coli communior B. paracoli

the surface of the flies and also within the intestines. The higher temperatures not only stimulate the flies to greater activity but also probably cause an increased bacterial development in the organic matter which they frequent. Irrespective of temperature, however, it was found throughout the summer that periods of richness of bacterial contamination would alternate with periods in which the flies were comparatively free from such pollution; and as a rule these lower counts on the surface of the insects were paralleled by a similar decrease for the intestinal content. Although the number of examinations were too few for a definite conclusion, it would seem that these fluctuations were due to the advent at regular intervals of swarms of newly hatched flies which had not an opportunity to infest the filth which constitutes the favorite pabulum of these household pests.

The figures of the table show that the surface contamination of these "wild" flies may vary from 570 to 4,400,000 bacteria per insect, and the intestinal bacterial content from 16,000 to 28,000,000. Taken as a whole the relation of the surface to the intestinal count was at the ratio of 1 to 8.6. It is probable that there is little or no increase in the number of fecal bacteria within the intestine of the fly and that no distinctive flora is developed as in warm-blooded animals. Accordingly the number of bacteria found constitute practically the sum total taken up with the insect's food.

Fecal bacteria of the colon group began to appear in notable numbers the last of June and at times were by far the dominant types, whereas at other times they entirely dropped out. An average of 10 days in which the counts were complete, excluding the excessively high count of July 13, showed that for the surface of the fly these colon group bacteria constituted 13.1 per cent of the total, with limits of none to 63 per cent. In the intestine the average was 37.5 per cent, with limits of none to 68 per cent of total count. This last average percentage of colon bacilli corresponds quite closely with the 39 per cent which Esten and Mason,[1] in one of the very few investigations bearing on this point, found to obtain for a large number of flies caught in the neighborhood of swill-barrels, stables, and within houses.[1] Of 39 cultures of lactose fermenters isolated and identified, 31, or 79.5 per cent, belonged within the colon-aerogenes group, and eight, or 20.5 per cent, were *B. acidi lactici.* In detail these 39 cultures were distributed as follows: *B. coli communis*, 10; *B. coli communior*, four; *B. aerogenes*, 17; *B. lactici*, eight. Esten and Mason also found that in their series the colon-aerogenes types predominated over the more innocent lactici-acidi type.

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[1] *Bull. 15, Agric. Exper. Station at Storrs, Conn.*, 1908.

Of the 15 cultures of streptococci isolated, none was *Str. pyogenes.* To a certain extent the favorite breeding place of flies, viz., horse manure, is revealed by the presence of *Str. equinus* on the surface of the flies, and their selected food by the *fecalis* and *salivarius* forms especially characteristic of human dejecta and frequently encountered in the intestinal content of the insects. One streptococcus culture split inulin but in no other respect resembled the pneumococcus.

The most noteworthy finding was that of three cultures of *B. paratyphus*, Type A, on plates made from the intestinal contents of flies caught on July 25. It is quite possible that more cultures of this bacillus might have been isolated from these plates if they had not been discarded before the nature of the bacilli was realized. At the time the cultures did not grow typically in the glucose gelatin agar tube medium, showing neither motility nor gas formation even when the medium was stirred, and in these respects simulating the dysentery bacillus. Subsequent tests, however, showed that they were typical paratyphoid bacilli in that they produced gas in dextrose, galactose, levulose, maltose, and mannite broth, acid but no gas in dextrin, and neither acid nor gas in lactose and saccharose media. Gelatin was not liquefied. They formed no indol in Dunham's peptone medium and gave rise to a typical fluorescence in neutral red glucose agar. In litmus milk they produced a slight acidity which continued for over two weeks and then gradually returned to the neutral point in two cultures and to a rather marked alkalinity in the third. That they belonged to the Type A group was shown by the following agglutination tests.[1]

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[1] Since this paper was sent to press my attention has been called to a recent article by William Nicoll (*Jour. Hyg.*, 1911, 11, p. 381). The writer has investigated the types of bacteria occurring naturally both within the intestine and on the surface of house flies caught in dwelling rooms and outhouses. He isolated 27 different varieties of the colon bacillus. He found, in agreement with my results, that the colon bacilli of the communis and communior types occurred far more frequently than *B. acidi lactici.* He recovered *B. paratyphosus*, Type B, from two flies, apparently infected outside the laboratory. He considers it more than likely that a considerable proportion of the bacteria in the intestine of the fly are simply passing through and never become resident, the colon bacilli tending to disappear and to be replaced by other organisms, frequently non-lactose-fermenting.

TABLE 2.

AGGLUTINATION OF FLY PARATYPHOID CULTURES WITH ANTI-PARATYPHOID SERUMS.

PARATYPHOID SERUMS	CULTURES					
	"Fly" 50	"Fly" 53	"Fly" 56	116	A	B
1. Anti-116	5,000	300	300	5,000	2,000	50−
2. Anti-A (16)	500	100	100	200	8,000	50−
3. Anti-B (22)	50+	50−	50−	50−	500	16,000

Absorption experiments, which are the true tests of specificity, revealed the interesting fact that although one of these cultures agglutinated to the limit with the anti-A serum yet it absorbed none of the specific agglutinin for the culture A. On the other hand these cultures absorbed completely the specific agglutinin for culture 116 in its antiserum. In these absorptions the serums diluted 1–10 were treated in each instance with the 24-hour growth from four agar cultures. This was sufficient in each instance to exhaust almost or quite completely the agglutinin for the culture used in the absorption.

TABLE 3.

ABSORPTION OF ANTI-116 PARATYPHOID SERUM.

CULTURES	WITH FLY CULTURE 50		WITH FLY CULTURE 56	
	Before	After	Before	After
Fly 50	5,000	20+	5,000	500
Paratyphoid A	2,000	500	2,000	1,000
Paratyphoid 116	5,000	20+	5,000	20−
Fly 56	.....	.....	300	20

ABSORPTION OF ANTI-A PARATYPHOID SERUM.

CULTURES	WITH FLY CULTURE 50		WITH FLY CULTURE 56	
	Before	After	Before	After
Fly 50	500	20−	500	20−
Paratyphoid A	8,000	8,000	8,000	8,000
Fly 56	.....	.....	100	20−

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[1] For the paratyphoid cultures A and B, I am indebted to the Department of Public Health of the American Museum of Natural History. These cultures reacted typically in litmus milk. The culture 116 was isolated in 1905 by Dr. Buxton from the blood of a typhoidal case in Bellevue Hospital. The antiserums were produced with rabbits.

Inoculations into guinea-pigs of these fly paratyphoid cultures disclosed approximately the same degree of toxicity as the stock paratyphoid cultures. Feeding experiments with white mice resulted negatively.

The similarity of these paratyphoid cultures isolated from flies to our stock culture 116 raises the question whether the insects picked up the bacilli inside or outside the laboratory. No absolute proof on one side or the other can be offered, but the probabilities are very strongly against the flies having gained access to this culture, as more than the ordinary precautions were taken against such a contingency. The stock cultures are kept habitually in the ice-box and the rabbits were inoculated with emulsions of the bacilli killed by heating. It would seem then that beyond a reasonable doubt these paratyphoid bacilli were introduced into the laboratory by the flies from some outside source.

Since the discovery of the group of bacteria intermediate between the colon and the typhoid forms, a great deal of investigation has been devoted to the occurrence and distribution of these types. It has been found that members of the Paratyphoid B or hog cholera group may be the causative agents in a wide range of animal diseases, among which are mouse typhoid, cat and rat enteritis, parrot and sparrow enteritis, swine pest, calf diarrhea, mastitis and enteritis of cows. In man, aside from their elimination by individuals clinically sick with paratyphoid fever, Prigge and Sachs-Muke[1] found in an examination of the dejecta of over 5,000 persons, 70 apparently healthy eliminators of paratyphoid bacilli. Further, they determined that these healthy carriers may be divided into two sharp groups. First, individuals who might have been in association with cases or might have become carriers through sickness. In one case a woman, who was an eliminator of paratyphoid bacilli during one and one-half years, finally developed severe clinical symptoms of paratyphoid infection. In the second group were placed the individuals never in contact with paratyphoid infection. This group included 14 women, 16 men, and 25 children. Among these the paratyphoid bacillus was found only once in repeated examinations, doubtless indicating that the bacilli were ingested with food and at once eliminated, the time coinciding with the examination. In this second group also belong the positive findings of Rimpau[1] in three of 50 school children, and the positive results of Mayer[2] in a similar investigation of the dejecta of sound children. Mayer states that the paratyphoid bacillus was found viable in dried feces after one and one-half years. Seiffert,[3] Dorset,[4] Uhlenhuth,[5] and others have found the hog cholera bacillus in the intestines of sound swine. These several references to the literature have been made to emphasize the fact that members of this group of bacilli intermediate between the colon and the typhoid bacillus are by no means confined to the clinically sick; accordingly there is the greater probability that dejecta or other matter containing them may at times be exposed to flies.

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[1] *Klin. Jahrb.*, 1910, 22, p. 237.

The group of paratyphoid bacilli known as Type A, to which agglutination experiments indicate the fly cultures belong, occurs less frequently in nature than Type B. Aside from its presence in typhoidal cases and enteritis, Uhlenhuth reports its isolation from a normal young pig and Schone[6] from sausage meat which apparently had given rise to an acute attack of vomiting and diarrhea. Ruediger[7] isolated a paratyphoid-like bacillus from the heart blood of a dog whose brain was examined for Negri bodies. This bacillus, though culturally like paratyphoid, agglutinated alone in a serum immune to Type A and with that at no higher dilution than 1–200. Apparently the present instance is the first in which paratyphoid bacilli have been isolated from flies.

It is noteworthy that the three cultures isolated are not identical as regards their agglutinability nor in their action on litmus milk. It is not to be inferred from this fact that they represent different strains of this micro-organism, because, as will be indicated elsewhere, they fall within the limits of possible variants in certain paratyphoid cultures. It is probable that they were simultaneously picked up from the same material. What this material may have been can only be surmised, but it seems probable that it was the dejecta either of a paratyphoid case or more likely that of a chronic carrier of this bacillus. That the culture is pathogenic for man is indicated by its high degree of agglutinability in the serum immune to a strain isolated from the blood of a typhoidal case.

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[1] *Deutsche med. Wchnschr.*, 1908, 34, p. 1045.

[2] *Centralbl. f. Bakt.*, I, Orig., 1910, 53, p. 234.

[3] *Ztschr. f. Hyg.*, 1909, 63, p. 273.

[4] *U.S. Dept. Agriculture, Bull.* 2, 1905.

[5] *Arb. a.d.k. Gsndhtsamte.*, 1908, 27, p. 425.

[6] *Ztschr. f. Hyg.*, 1910, 65, p. 1.

[7] *Jour. Infect. Dis.*, 1911, 8, p. 486.

It would seem reasonable that not alone the bacilli on the surface of the flies should be taken into consideration in passing judgment on these insects as disease-spreaders, but also those within the intestinal tract, as flies are continually depositing their excreta on dishes used for food and on food itself.[1]

The bacilli designated *B. paracoli* in Table 1 are also deserving of special comment. Certain of these cultures when first isolated were strikingly like the dysentery bacillus except that they produced a little more luxuriant growth on glycerin agar than the dysentery form. They showed no visible gas in Hiss semi-solid tube medium even when vigorously stirred and caused no outgrowth into the medium after an incubation of several weeks. They split the monosaccharids alone with the formation of acid, but not mannite, maltose, lactose, dextrin, or saccharose. In litmus milk there occurred a primary acidity with a bluish-green cream which gave way in four or five days to a final moderate alkalinity. They would not agglutinate, however, in serums immune to the Shiga and Y types of the dysentery bacillus and, except for one culture with which there was a positive reaction at 1–200, reacted negatively with the Flexner type serum. Subsequent tests, however, showed that these cultures produce a moderate amount of gas with dextrose and galactose broth in fermentation tubes. Apparently the Hiss semi-solid tube medium cannot be depended upon to reveal the gas-producing properties of certain non-motile bacilli. The greater number of the isolations of these bacilli, however, produced after three days incubation a clouding of the tube medium of varying degree, either in the form of a diffuse cloud or an outgrowth from the stab, but without gas formation. In litmus milk the primary acidity gave way to a final intense blue-black alkalinity. None of these cultures liquefied gelatin. Duval and Schorer[2] in their report on the bacteriology of the summer diarrhea of infants described this bacillus as occurring quite frequently in the stools, and comment on its somewhat superficial resemblance to the dysentery bacillus. They apparently did not ascertain that these bacilli will form gas in dextrose broth and that they one and all produce indol in peptone water and also a yellowish or greenish fluorescence with gas in neutral red dextrose agar. Evidently this bacillus has not been recognized heretofore as belonging in the group of paracolon bacilli.[1] They comprise a fluctuating family in which the visible gas-producing property is frequently suppressed. For guinea-pigs they exhibit a pathogenicity fully equal to that of *B. coli communis* or *B. paratyphosus*.

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[1] According to the findings of G. S. Graham-Smith (*Rep. to the Loc. Gov. Bd. on Public Health and Med. Subjects*, 1910, N.S., 40, p. 1) bacteria on the surface of the fly quickly die, whereas pathogenic bacteria which have been taken into the digestive tract may continue there in a viable condition for some time and gradually be eliminated. For this reason he considers the intestinal flora of more importance from a sanitary standpoint than that on the surface of the insect.

[2] *Studies from the Rockefeller Institute*, 1904, 2, p. 42.

A culture of a diphtheroid bacillus which was isolated from flies the last of August is also of interest, in that the bacilli were morphologically identical with certain granular forms of the Klebs-Loeffler bacillus. These bacilli when stained with Neisser's method exhibit blue-black dots, often occurring at each end of the bacillus, which were typically barred and not uniform in length. Here, however, the resemblance to true diphtheria bacilli ended, as this culture produced a thick staphylococcus-like growth on glycerin agar and elaborated no toxin in alkaline sugar-free broth. They resembled *B. xerosis* in splitting saccharose with acid production but not dextrin; accordingly they have been so designated.

SUMMARY.

1. Flies examined up to the latter part of June were free from fecal bacteria and carried a homogeneous flora of coccal forms.

2. During July and August there occurred periods in which the flies examined carried several millions of bacteria, alternating with periods in which the number of bacteria were reduced to hundreds. The scanty flora probably indicated the advent of swarms of recently hatched flies.

3. Fecal bacteria of the colon type were first encountered in abundance the early part of July.

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[1] Probably certain, at least, of these bacilli are identical with that described by H. deR. Morgan and J. C. G. Ledingham (*Proc. Roy. Soc.*, 1909, Med. II, Part II, p. 133) and named by them "Morgan's bacillus." They consider this bacillus an important factor in the summer diarrheas of children, chiefly because of its occurrence in 63 per cent of selected cases. They were able to isolate this bacillus from flies caught in both infected and uninfected houses.

4. The bacteria in the intestines of the flies were 8.6 times as numerous as on the surface of the insects.

5. On the surface of the flies the colon group bacteria constituted 13.1 per cent of the total; and within the intestine 37.5 per cent of the total.

6. Of the lactose fermenters, isolated and identified, 79.5 per cent belonged in the colon-aerogenes group and 20.5 per cent in the acidi lactici group.

7. Fifteen cultures of streptococci, isolated and identified, were distributed among the equinus, fecalis, and salivarius groups. There were none of the pyogenes type.

8. The most important isolations were three cultures of *B. paratyphosus*, Type A.

9. Bacteria of the paracolon type causing a final intense alkaline reaction in litmus milk and fermenting only certain monosaccharids were frequently encountered during August.